

Complete Genome Sequence of *Mycoplasma californicum* Strain HAZ160_1 from Bovine Mastitic Milk in Japan

Eiji Hata,^a Kenji Murakami^b

Dairy Hygiene Research Division, National Institute of Animal Health (NIAH), National Agriculture and Food Research Organization (NARO), Sapporo, Hokkaido, Japan^a; Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan^b

Bovine mycoplasmal mastitis is spreading quickly among cows. It often leads to clinical mastitis outbreaks and often results in huge economic losses. *Mycoplasma californicum* is an important causal species of bovine mastitis. Presented here is the 799,088-bp complete genome sequence of *M. californicum* strain HAZ160_1, which was isolated in Japan.

Received 16 June 2014 Accepted 23 June 2014 Published 10 July 2014

Citation Hata E, Murakami K. 2014. Complete genome sequence of *Mycoplasma californicum* strain HAZ160_1 from bovine mastitic milk in Japan. *Genome Announc.* 2(4): e00684-14. doi:10.1128/genomeA.00684-14.

Copyright © 2014 Hata and Murakami. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to Eiji Hata, ehata@affrc.go.jp.

Mycoplasma californicum is a causal bacterium of bovine mastitis, arthritis, and pneumonia as well as an indigenous bacterium affecting cattle (1, 2). Mastitis due to *M. californicum* features strong infectivity, severe symptoms, and poor response to treatment with antibiotics and is often accompanied by major economic losses (2–4). Despite its importance, little genetic information on *M. californicum* is available. Presented here is the whole-genome sequence of strain HAZ 160_1, which was isolated in 2008 from bovine mastitic milk in Japan.

Total genomic DNA was prepared from *M. californicum* strain HAZ 160_1 and subjected to 454 Titanium sequencing at the Hokkaido System Science Co., Ltd., Sapporo, Japan. The resulting reads were assembled *de novo* using GS *de novo* Assembler software version 2.7 (Roche), yielding 36 contigs with 94.8× coverage. An analysis of the contig ends together with PCR amplification and amplicon cloning showed that the 799,088-bp genome had a closed-ring structure. After the initial automated annotation performed using the Microbial Genome Annotation Pipeline version 2.18 at the DNA Data Bank of Japan (<http://mgap.ddbj.nig.ac.jp/mgap/jsp/index.jsp>) (5–7), manual curation was performed, followed by verification of potential pseudogenes by PCR and Sanger sequencing. As a result, we confirmed 574 open reading frames, 15 pseudogenes, 31 tRNAs, and 2 sets of each rRNA (5S rRNA, 16S rRNA, and 23S rRNA) in this genome sequence. Moreover, the G+C content was 30.8%.

As anticipated based on its 16S rRNA-based phylogeny, most genes in *M. californicum* strain HAZ 160_1 exhibited high similarity to the amino acid sequences of the genes encoding members of the *Mycoplasma fermentans* cluster, with the greatest similarity shown with genes from the bovine pathogen *Mycoplasma bovis* (8).

The hypothetical proteins MCAL160_0738, MCAL160_0902, MCAL160_0908, and MCAL160_0912 may be involved in the antigenic variation shift in surface proteins that plays a role in the adaptation to new surroundings and in host defense mechanisms (9). A part of the amino acid sequences of these genes showed certain similarity to the membrane proteins of other *Mycoplasma*

and *Ureaplasma* species. Moreover, the discriminative homopolymeric tract of contiguous thymines (Poly-T) is located upstream of repetitive regions in the hypothetical proteins MCAL160_0738, MCAL160_0908, and MCAL160_0912 (9). These genes contain repetitive regions encoding distinctive periodic amino acid sequences, and the number of repetitive units differed among *M. californicum* strains.

Although this genome sequence contained genes of proteins involved in the synthesis of capsular polysaccharides, which are suggested to be important mycoplasmal etiologic agents, i.e., UTP-glucose-1-phosphate-uridylyltransferase and glycosyltransferases, etc., the genes of proteins involved in the production of active oxygen-containing molecules, which are also suggested to be important etiologic agents, were not confirmed (10).

The genomic sequence of *M. californicum* will provide a foundation for the investigation of this species in the future. Ultimately, it is hoped that this study will contribute to the reduction of bovine diseases such as mastitis or pneumonia.

Nucleotide sequence accession number. This whole-genome sequence has been registered at DDBJ/EMBL/GenBank under accession no. [AP013353](https://www.ncbi.nlm.nih.gov/nuccore/6013353).

ACKNOWLEDGMENTS

This research was supported by grants from the Ministry of Agriculture, Forestry and Fisheries of Japan and the National Agriculture and Food Research Organization (NARO) of Japan.

REFERENCES

- Hewicker-Trautwein M, Feldmann M, Kehler W, Schmidt R, Thiede S, Seeliger F, Wohlsein P, Ball HJ, Buchenau I, Spergser J, Rosengarten R. 2002. Outbreak of pneumonia and arthritis in beef calves associated with *Mycoplasma bovis* and *Mycoplasma californicum*. *Vet. Rec.* 151:699–703. <http://dx.doi.org/10.1136/vr.151.23.699>.
- Jasper DE. 1982. The role of *Mycoplasma* in bovine mastitis. *J. Am. Vet. Med. Assoc.* 181:158–162.
- Ball HJ, Campbell JN. 1989. Antibiotic treatment of experimental *Mycoplasma californicum* mastitis. *Vet. Rec.* 125:377–378. <http://dx.doi.org/10.1136/vr.125.14.377>.
- Mackie DP, Ball HJ, Logan EF. 1986. *Mycoplasma californicum* mastitis

- in the dry dairy cow. *Vet. Rec.* 119:350–351. <http://dx.doi.org/10.1136/vr.119.14.350>.
5. Noguchi H, Taniguchi T, Itoh T. 2008. MetaGeneAnnotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes. *DNA Res.* 15: 387–396. <http://dx.doi.org/10.1093/dnares/dsn027>.
 6. Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 35:3100–3108. <http://dx.doi.org/10.1093/nar/gkm160>.
 7. Tatusov RL, Fedorova ND, Jackson JD, Jacobs AR, Kiryutin B, Koonin EV, Krylov DM, Mazumder R, Mekhedov SL, Nikolskaya AN, Rao BS, Smirnov S, Sverdlov AV, Vasudevan S, Wolf YI, Yin JJ, Natale DA. 2003. The COG database: an updated version includes eukaryotes. *BMC Bioinformatics* 4:41. <http://dx.doi.org/10.1186/1471-2105-4-41>.
 8. Pettersson B, Uhlén M, Johansson KE. 1996. Phylogeny of some mycoplasmas from ruminants based on 16S rRNA sequences and definition of a new cluster within the hominis group. *Int. J. Syst. Bacteriol.* 46: 1093–1098. <http://dx.doi.org/10.1099/00207713-46-4-1093>.
 9. Razin S, Yogev D, Naot Y. 1998. Molecular biology and pathogenicity of mycoplasmas. *Microbiol. Mol. Biol. Rev.* 62:1094–1156.
 10. Westberg J, Persson A, Holmberg A, Goesmann A, Lundeberg J, Johansson KE, Pettersson B, Uhlén M. 2004. The genome sequence of *Mycoplasma mycoides* subsp. *mycoides* SC type strain PGIT, the causative agent of contagious bovine pleuropneumonia (CBPP). *Genome Res.* 14: 221–227. <http://dx.doi.org/10.1101/gr.1673304>.